#5

Fig. 1

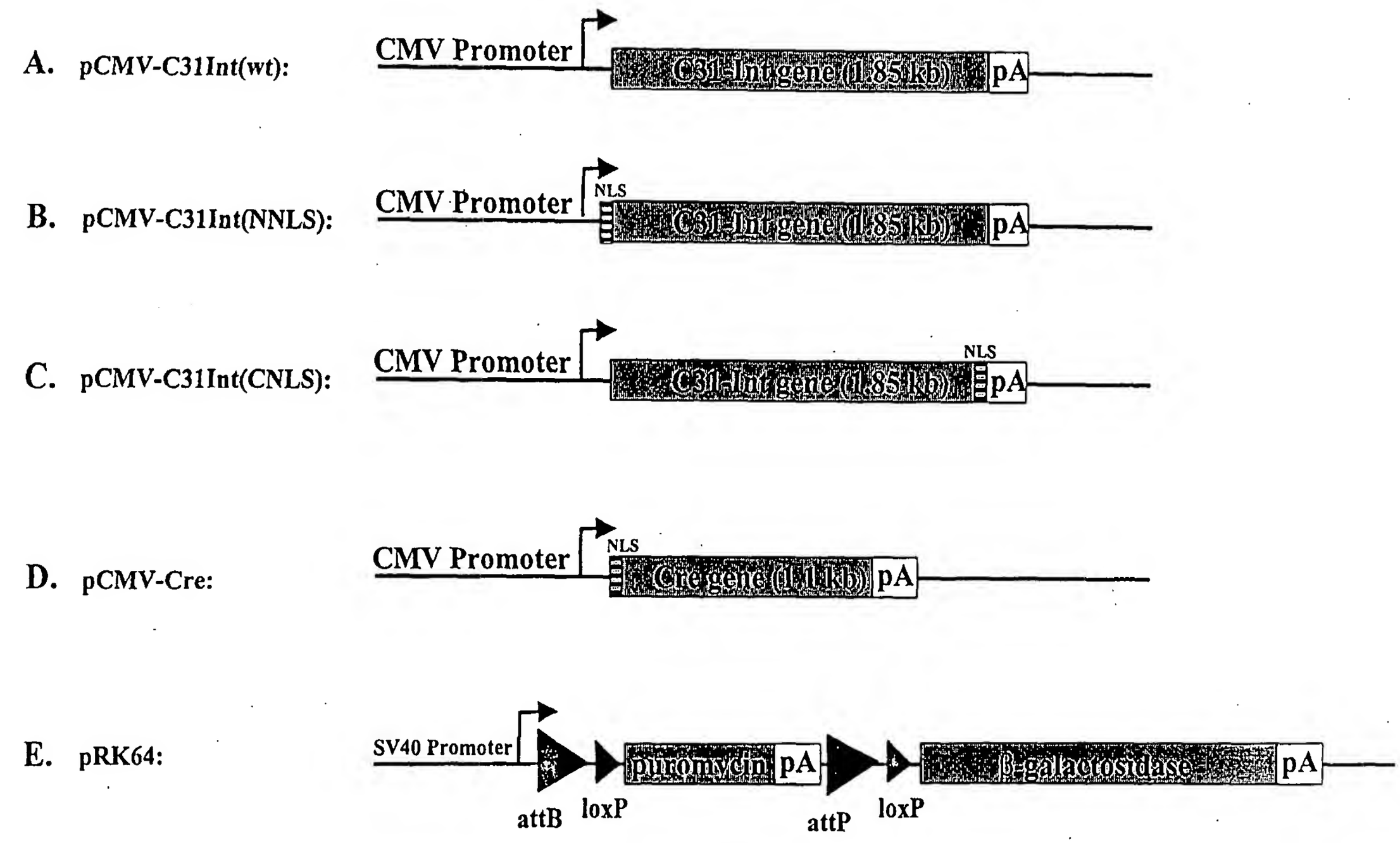

A. pCMV-C31Int(wt):
CMV Promoter
C31-Int gene (1.85 kb)
pA
B. pCMV-C31Int(NNLS):
NLS
CMV Promoter
C31-Int gene (1.85 kb)
pA
C. pCMV-C31Int(CNLS):
CMV Promoter
C31-Int gene (1.85 kb)
NLS
pA
D. pCMV-Cre:
NLS
CMV Promoter
Cre gene (1.1 kb)
pA
E. pRK64:
SV40 Promoter
puromycin
pA
β-galactosidase
pA
attB
loxP
attP
loxP

Fig. 2

| Sample | RLU (βGal) | RLU (Luciferase) | RLU x10$^5$ (Gal/Luc) | RLU x10$^5$ (Gal/Luc) | relative activity |
|---|---|---|---|---|---|
| | | | | $10^4$ 5 x $10^4$ | |
| 1) pUC19 only | 433 ± 37 | 1818700 ± 328970 | **24** ± 4 | | |
| 2) pRK64(ΔCre) | 784932 ± 290524 | 1657062 ± 526562 | **46975** ± 3696 | | 1 |
| 3) pRK64 only | 1936 ± 262 | 988144 ± 175116 | **204** ± 62 | | 0.004 |
| 4) pCMV-C31Int(wt) 0.5 ng | 73318 ± 19084 | 677861 ± 145341 | **10774** ± 972 | | 0.23 |
| 5) pCMV-C31Int(wt) 1 ng | 75838 ± 12628 | 527237 ± 53846 | **14412** ± 2050 | | 0.3 |
| 6) pCMV-C31Int(NNLS) 0.5 ng | 158402 ± 75870 | 2560450 ± 736186 | **15104** ± 3041 | | 0.32 |
| 7) pCMV-C31Int(NNLS) 1 ng | 206857 ± 76733 | 2677621 ± 504285 | **17029** ± 2246 | | 0.36 |
| 8) pCMV-C31Int(CNLS) 0.5 ng | 274192 ± 78937 | 1173932 ± 291315 | **23299** ± 3194 | | 0.5 |
| 9) pCMV-C31Int(CNLS) 1 ng | 262169 ± 60583 | 864752 ± 229935 | **30560** ± 1585 | | 0.65 |
| 10) pCMV-Cre(NNLS) 0.5 ng | 231200 ± 96741 | 763121 ± 280687 | **29595** ± 4632 | | 0.63 |
| 11) pCMV-Cre(NNLS) 1 ng | 297760 ± 83363 | 868905 ± 196404 | **33872** ± 2609 | | 0.72 |

**Fig. 3**

| | RLU (βGal) | RLU (Luciferase) | RLU x $10^5$ (Gal/Luci) |
|---|---|---|---|
| 1) pPGKnifD (reporter) only | 1324 ± 876 | 3631598 ± 903012 | 34 ± 18 |
| 2) pCMV-XisA 25 ng | 4650 ± 2273 | 2741969 ± 667568 | 164 ± 54 |
| 3) pCMV-XisA 100 ng | 17529 ± 9304 | 3798872 ± 1288020 | 443 ± 151 |
| 4) pCMV-XisA(NNLS) 25 ng | 4060 ± 1376 | 2471695 ± 611351 | 163 ± 36 |
| 5) pCMV-XisA(NNLS) 100 ng | 17801 ± 3892 | 3570103 ± 750628 | 500 ± 65 |
| 6) pPGKattA (reporter) only | 754 ± 70 | 195822 ± 81858 | 755 ± 601 |
| 7) pCMV-SSV 10 ng | 925 ± 273 | 119043 ± 67451 | 906 ± 316 |
| 8) pCMV-SSV 20 ng | 1033 ± 270 | 122557 ± 30054 | 879 ± 291 |
| 9) pCMV-SSV(NNLS) 10 ng | 1108 ± 367 | 174380 ± 58876 | 694 ± 345 |
| 10) pCMV-SSV(NNLS) 20 ng | 1306 ± 383 | 211182 ± 101011 | 874 ± 741 |

**Fig. 4**

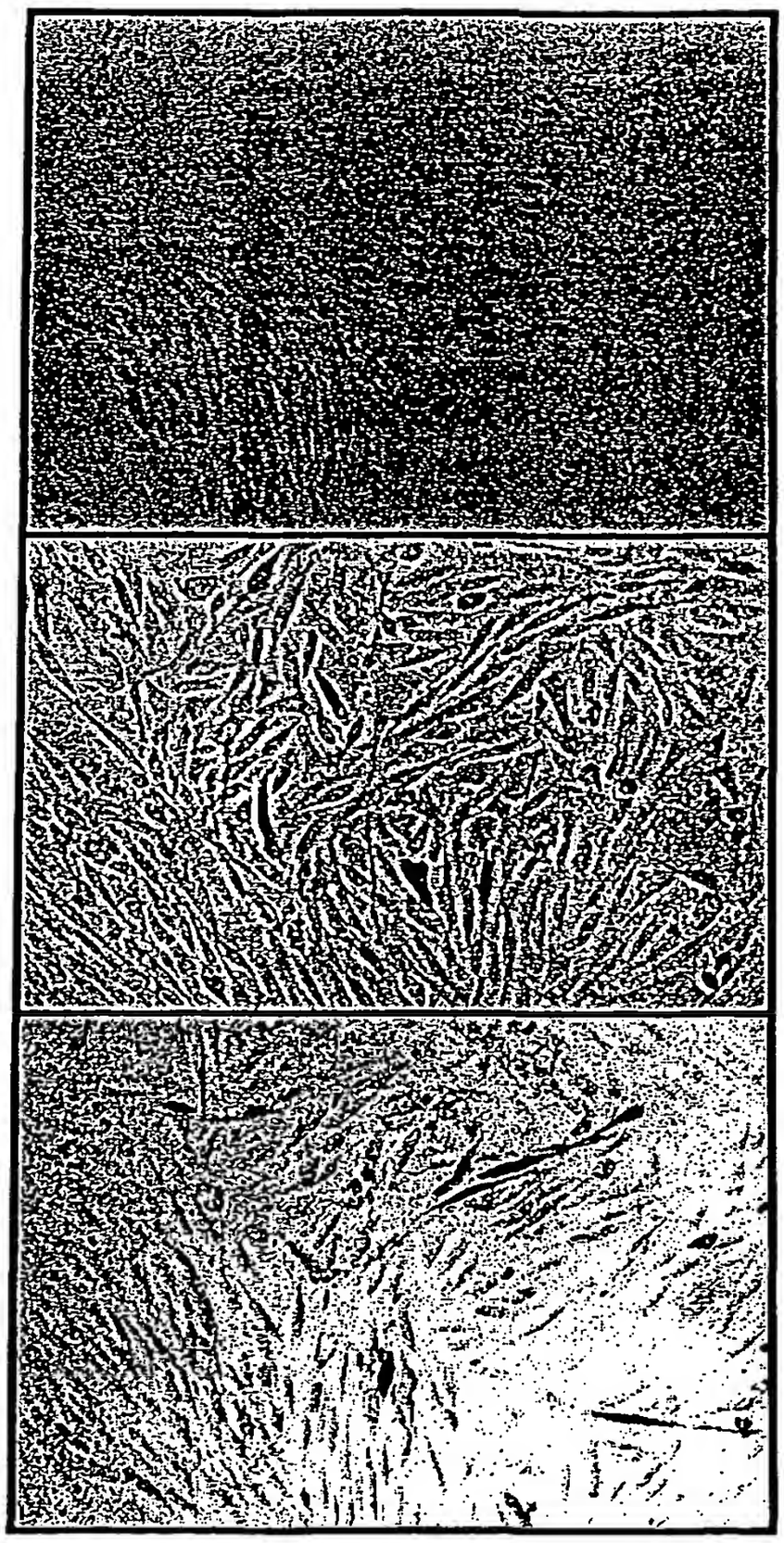
| Sample | RLU (βGal) | RLU (βGal) | relative activity |
|---|---|---|---|
| | | 5 x $10^3$ — 4 x $10^4$ | |
| 1) pUC19 only | 754 ± 44 | | |
| 2) pCMV-C31Int(wt) 32 ng | 1386 ± 174 | | 0.04 |
| 3) pCMV-C31Int(wt) 64 ng | 3783 ± 1537 | | 0.1 |
| 4) pCMV-C31Int(NNLS) 32 ng | 7125 ± 1474 | | 0.19 |
| 5) pCMV-C31Int(NNLS) 64 ng | 8206 ± 2210 | | 0.22 |
| 6) pCMV-C31Int(CNLS) 32 ng | 17624 ± 5578 | | 0.48 |
| 7) pCMV-C31Int(CNLS) 64 ng | 28849 ± 6623 | | 0.78 |
| 8) pCMV-Cre(NNLS) 32 ng | 27064 ± 3769 | | 0.73 |
| 9) pCMV-Cre(NNLS) 64 ng | 36823 ± 3993 | | 1 |

Fig. 5

A.: Nontransfected control

B.: pCMV-Cre

C.: pCMV-C31Int(NLS)



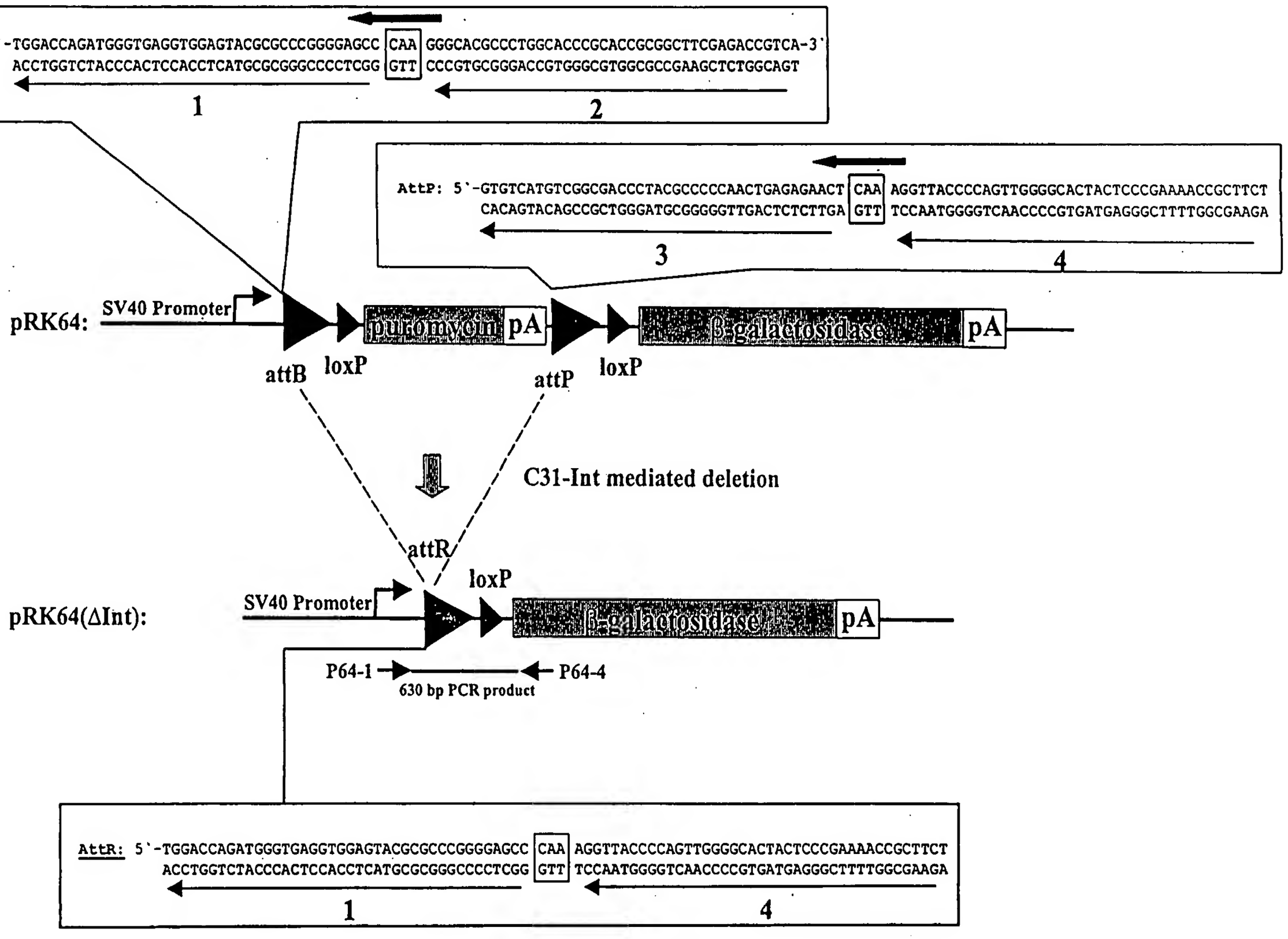
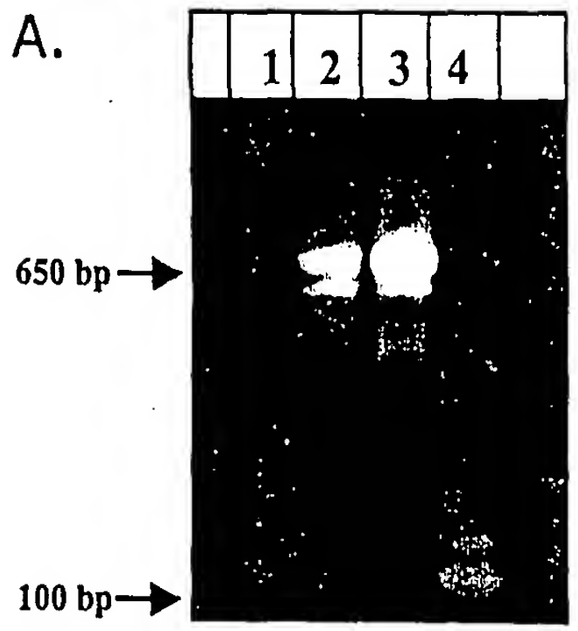

AttB: 5`-TGGACCAGATGGGTGAGGTGGAGTACGCGCCCGGGGAGCC CAA GGGCACGCCCTGGCACCCGCACCGCGGCTTCGAGACCGTCA-3`
ACCTGGTCTACCCACTCCACCTCATGCGCGGGCCCCTCGG GTT CCCGTGCGGGACCGTGGGCGTGGCGCCGAAGCTCTGGCAGT
1
2
AttP: 5`-GTGTCATGTCGGCGACCCTACGCCCCCAACTGAGAGAACT CAA AGGTTACCCCAGTTGGGGCACTACTCCCGAAAACCGCTTCT
CACAGTACAGCCGCTGGGATGCGGGGGTTGACTCTCTTGA GTT TCCAATGGGGTCAACCCCGTGATGAGGGCTTTTGGCGAAGA
3
4
pRK64:
SV40 Promoter
puromycin
pA
β-galactosidase
pA
attB
loxP
attP
loxP
C31-Int mediated deletion
attR
loxP
pRK64(ΔInt):
SV40 Promoter
β-galactosidase
pA
P64-1
P64-4
630 bp PCR product
AttR: 5`-TGGACCAGATGGGTGAGGTGGAGTACGCGCCCGGGGAGCC CAA AGGTTACCCCAGTTGGGGCACTACTCCCGAAAACCGCTTCT
ACCTGGTCTACCCACTCCACCTCATGCGCGGGCCCCTCGG GTT TCCAATGGGGTCAACCCCGTGATGAGGGCTTTTGGCGAAGA
1
4

Fig. 6

**Fig. 7**

A.



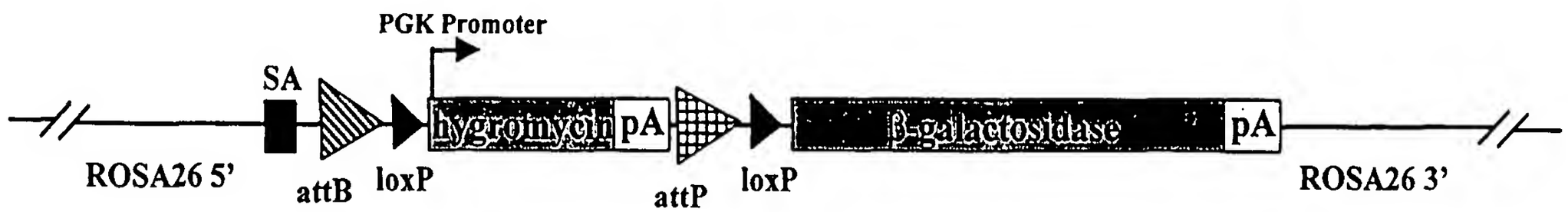

B.

attR

1 4

pRK64(Δint)(pos.335-437):5`-ccggtacagttcg tggaccagatgggtgaggtggagtacgcgcccggggagcc caa aggttaccccagttggggcactactcccgaaaaccgcttct ggatcc-
Seq80d (pos.350-452):5`-ccggtacagttcg tggaccagatgggtgaggtggagtacgcgcccggggagcc caa aggttaccccagttggggcactactcccgaaaaccgcttct ggatcc-

Fig. 8


PGK Promoter
SA
hygromycin
pA
β-galactosidase
pA
ROSA26 5'
attB
loxP
attP
loxP
ROSA26 3'

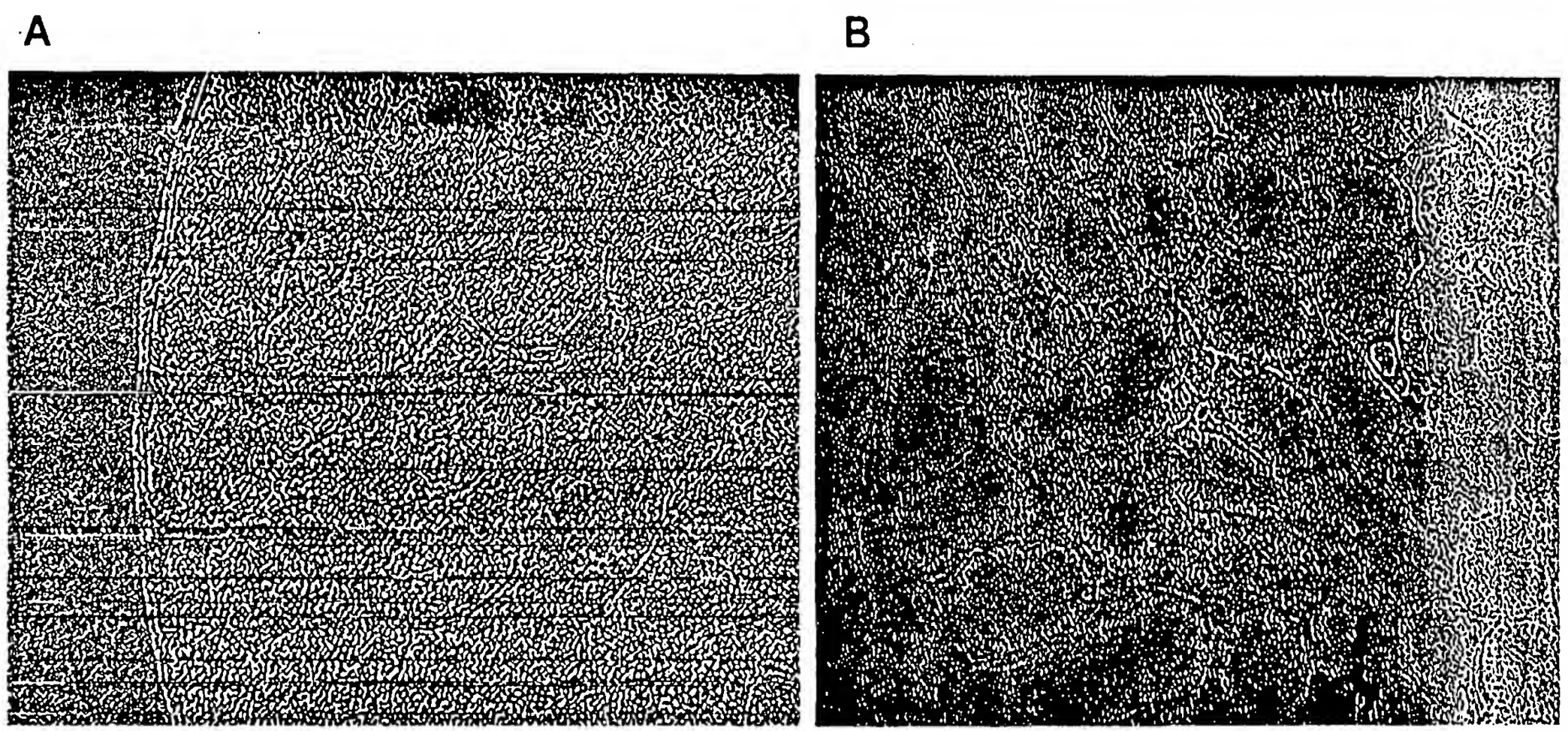



Fig. 9